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Viability of lactic acid bacteria in different components of *Ogi* with anti diarrhoeagenic *E. coli* activities

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ABSTRACT

Background: *Ogi* constitutes a rich source of lactic acid bacteria (LAB) with associated health benefits to humans through antimicrobial activities. However, the high viability of LAB in *Ogi* and its supernatant (*Omidun*) is essential. **Aims:** This study was carried out to assess the viability of LAB in various forms of modified and natural *Ogi* and the antimicrobial properties of *Omidun* against diarrhoeagenic *E. coli*. **Methods and Material:** The viability of LAB was assessed in fermented *Ogi* slurry and *Omidun* for one month and also freeze-dried *Ogi* with and without added bacterial strains for two months. A further 10 days viability study of modified *Omidun*, refrigerated *Omidun*, and normal *Ogi* was performed. The antimicrobial effects of modified *Omidun* against five selected strains of diarrhoeagenic *E. coli* (DEC) were evaluated by the co-culture method. **Results:** Both drying methods significantly affected carotenoids and phenolic compounds. The *Ogi* slurry had viable LAB only for 10 days after which, there was a succession of fungi and yeast. *Omidun* showed 2 log₁₀cfu/ml reduction of LAB count each week and the freeze-dried *Ogi* showed progressive reduction in viability. Refrigerated *Omidun* has little viable LAB, while higher viability was seen in modified *Omidun* (≥ 2 log cfu/ml) than normal *Omidun*. Modified *Omidun* intervention led to 2-4 log reduction in diarrhoeagenic *E. coli* strains and total inactivation of shigella-toxin producing *E. coli* H66D strain in co-culture. **Conclusions:** The consumption of *Ogi* should be within 10 days of milling using modified *Omidun*. There are practical potentials of consumption of *Omidun* in destroying *E. coli* strains implicated in diarrhea.

Keywords: *Ogi*, *Omidun*, lactic acid bacteria, diarrhoeagenic *Escherichia coli* strains, Viability.

1 Introduction

Fermentation preserves foods by converting carbohydrates to alcohol and organic acids [1]. Microbes such as lactic acid bacteria (LAB) are involved in natural fermentation processes that produce fermented foods. [2,3]. The influences of the fermentation microbes on the nature of the food and their antimicrobial properties are well characterized [4-6]. Aderiyi *et al.*, [7] described the use of fermented cereals as foods with enhanced health properties e.g. hypolipidemic, hepatoprotective, antibacterial, and treatment of gastroenteritis in man and animals.

WHO [8] described probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" i.e. viability and consumption of sufficient numbers are an inherent property of probiotics. One of the best uses for probiotics is the reduction of infectious diarrhea and diarrhea associated with antibiotic use. Probiotics shorten diarrhea episodes. Diarrhoeagenic *Escherichia coli* strains are among the commonest causative agents of diarrhea and are divided into enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli*

(ETEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC). Some developing countries, such as Nigeria, are still struggling against increasing morbidity and mortality of diarrheal infections in young children. Different home remedies are usually employed to combat diarrhea menace. The use of *Omidun* (the supernatant on *Ogi*) constitutes an example. *Ogi* is a fermented cereal gruel widely consumed in Western Nigeria in breakfast and in traditional infant weaning food [9]. Aderiye and Laleye, [10] stated that, although some communities in south-western Nigeria administered uncooked *Ogi* to people with diarrhea to reduce the frequency of stooling, the scientific proof for this claim is lacking. Several authors have described functional, nutritional and antibacterial properties of *Ogi* [11-15], but there is insufficient data on the viability of LAB in different components of *Ogi* over a period of time. Therefore, this study was designed to study the component of *Ogi* that has the most viable LAB over a period of time and the antimicrobial properties of *Omidun* against different strains of diarrhoeagenic *Escherichia coli*.

2 Material and Methods

2.1 Bacterial strains

2.1.1 Diarrhoeagenic *E. coli* strains

All diarrhoeagenic strains of *E. coli* were obtained from the Molecular Microbiology Unit, Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Nigeria. Five different strains of *Escherichia coli* (Enterohaemorrhagic *E. coli* (EAEC LLD25D), Enterotoxigenic *E. coli* (ETEC LWD21A), Shiga-toxin producing *E. coli* (STEC LLH74B), Enteroinvasive *E. coli* (EIEC LWD21E) and Enteropathogenic *E. coli* (EPEC LLH78D) were used for the modified *Omidun* co-culture experiment.

2.1.2 Lactobacilli strains

Two strains of already characterized LAB; *Lactobacillus paraplantarum* AI3, and *Lactobacillus pentosus* AO82 with good antimicrobial properties were obtained from the probiotic group of Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.

2.2 Traditional fermentation of *Ogi*

Maize grains (*Zea mays*) were obtained from the Bodija market, Ibadan Oyo State Nigeria. The *Ogi* was prepared by fermentation of maize grains according to the traditional methods of processing as described by Afolayan *et al.* [14]. In summary, Maize grains were soaked in clean water for 48 h at $28 \pm 2^\circ\text{C}$. The fermenting water was decanted and the soften maize grains were wet milled

using clean grinding machine. The paste obtained was sieved with a clean muslin cloth to remove the husks. The filtrate was allowed to settle and ferment according to the days subsequently described for each experiment. The filtrate separated into thick paste (*Ogi*) and watery supernatant (*Omidun*). The pH was evaluated for a period of 7 days.

2.3 *Ogi* and *Omidun* viability studies

On the first day of souring, 1g of *Ogi* slurry was obtained from the surface of *Ogi* (this involves lightly scrapping the surface of settled *Ogi* in order to obtain maximum bacterial counts) and 1 ml of *Omidun* were appropriately diluted with 0.9% sterile normal saline and the dilutions were inoculated on MRS agar respectively [14]. All incubations were carried under micro-aerophilic conditions at 37°C for 24-48 hours. The number of colony-forming units on the MRS agar were counted and identified by morphological characteristics, Gram reaction, and catalase test. The procedure was repeated weekly on *Ogi* slurry and *Omidun* for 26 days.

2.4 Freeze-dried *Ogi* powder viability study

The effect of freeze-drying on LAB viability in *Omidun* and milk was done according to a modified method of Ayeni *et al.* [16]. Grown 24 h cultures of *Lactobacillus paraplantarum* AI3 and *Lactobacillus pentosus* AO82 respectively were centrifuged at 3000 rpm for 10 mins, washed in normal saline and then resuspended in 0.5 mL *Omidun* and sterile milk respectively. The resuspended pellets of the LAB in *Omidun* was mixed with 10 g of wet *Ogi* slurry. Also the resuspended LAB in milk was mixed with 1 mL of milk for each sample. The viability counts of the mixtures were carried out before freeze-drying. All the five different components (*Ogi* alone, *Ogi* + *L. paraplantarum*, *Ogi* + *L. pentosus*, milk + *L. paraplantarum*, milk + *L. pentosus*) were collected and freeze-dried by freezing them to -20°C at atmospheric pressure then sublimed the frozen product at -20°C , which was then transferred to a condenser at low temperature and then defrost to yield a powdered product. The viability counts before and after freeze-drying and also after 69 days of storage at room temperature was performed for bacterial strains vehiculated in *Ogi* and milk. For *Ogi*, the viability at 26 days was measured and then discontinued due to low survival rate of LAB.

A study on the effects of capsulation on lactobacilli vehiculated in *Ogi* was adapted from the viability count method of Ayeni *et al.* [16]. Freeze-dried *Ogi* and milk were put in capsule shells, filled and spread over to ensure uniform filling of the capsules. The cap was fixed appropriately over the body and stored at room temperature. The viability counts of LAB in capsules versus the freeze-dried products that were not stored in capsules

for the *Ogi* products were done after three weeks of storage and the results recorded. The difference in the viability of LAB cryopreserved with milk *vs* *Ogi* was analyzed with student t-test.

2.5 Evaluation of the viability of LAB in *Ogi* over a period of ten days

The results obtained from the initially described viability study made us develop a new protocol to evaluate the maximum viability of *Ogi* components over 10 days. Freshly prepared *Ogi* with *Omidun* [14] were divided into three sterile containers. In the first container, *Omidun* was changed every day and viability study was done with mixture of *Omidun* (removed before changing the water) and lightly scrapped surface of *Ogi*. This mixture is tagged 'modified *Omidun*' and 1 ml of the mixture was serially diluted and plated out on MRS agar for viability counts after incubating micro aerophilically for 24-48 h. The procedure was repeated daily for 10 days. In the second container, *Ogi* was allowed to settle and *Omidun* changed daily. The surface of settled *Ogi* was lightly scrapped and 1 g obtained from the scrapped material was mixed in 9 mL of saline and diluted appropriately, then plated on MRS agar to get the viability counts daily for ten days. From the third container, *Omidun* was decanted after milling and settling, then kept in the fridge for 10 days. Analyses were done by daily removing 1 mL of the refrigerated *Omidun* and plating out as previously described for ten days.

2.6 Determination of the antimicrobial effect of modified *Omidun* on diarrhoeagenic *Escherichia coli*

The method of Ojo *et al.* [5] was used to study the antimicrobial effects of *Omidun* on diarrheagenic *E. coli* pathotypes. We used five different diarrheagenic *E. coli* strains: EAEC LLD25D, ETEC LWD21A, STEC LLH74B, EIEC LWD21E and EPEC LLH78D. The strains were grown for 24 h in Nutrient Broth and 0.1 mL of each *E. coli* strains were introduced into 99.9 mL of modified *Omidun* mixture as previously described. One milliliter from the mixture was diluted serially in 9 mL of normal saline and plated out on MacConkey agar to get the viable counts of the *E. coli* strains at time zero (0 h) by incubating for 24 h at 37°C. The remaining 99 mL mixture of modified *Omidun* and *E. coli* were incubated for 24 h at 37°C aerobically. One milliliter from the mixture was diluted serially in 9 mL of normal saline and plated out on MacConkey agar to get the viable counts of the *E. coli* strains at time 24 (24 h) by incubating for 24 h at 37°C. The control experiment involved growing the different *E. coli* strains in normal saline and plating out the viable cells at time 0 h and 24 h. The plates were then

observed for the growth of *E. coli* and viable colonies counted. The results were recorded at 0 h and 24 h.

3 Results

The pH of traditionally prepared *Ogi* was evaluated over 7 days as Day 1: 3.96, Day 2: 3.45, Day 3: 3.77, Day 4: 3.62, Day 5: 3.50 - Day 7: 3.98. The highest pH was 3.98 on day 7 and the lowest was 3.45 on day 2. The result of quantities of viable LAB in different components of *Ogi*: *Ogi* slurry; *Omidun*, modified *Omidun* and freeze-dried *Ogi* at different time intervals are shown on Figures 1 to 3. There was an increase in quantity of viable LAB in *Ogi* slurry as the number of days increases, ranging from 8.6×10^8 cfu/ml on day 1 of the souring period to one log increase (5.2×10^9 cfu/ml) on day 3 and further one log increase (9.7×10^9 cfu/ml) on day 10. However, after ten days, there was succession of fungi growth (Fig 1). The LAB present in *Omidun* showed viability for the four weeks duration, though with progressive reduction in quantities of viable LAB as the days from 1.25×10^7 cfu/ml on day 1 to 3.5×10^2 cfu/ml on day 27 (Fig. 1).

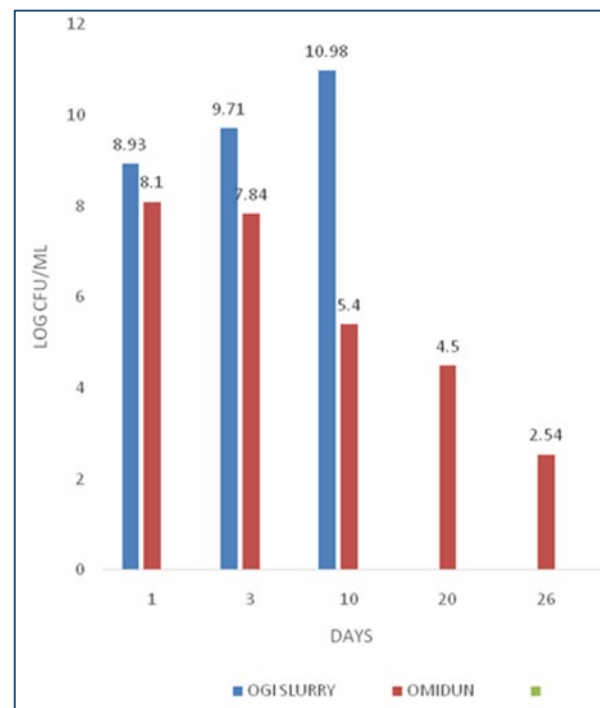


Figure 1: Viability of LAB in *Ogi* slurry and *Omidun*

In the 10 days viability study, LAB in *Omidun* remained viable over a period of 10 days. On the first day, modified *Omidun* had LAB counts of 6.2×10^7 CFU/ml, *Ogi* alone had 3.5×10^9 cfu/ml and refrigerated *Omidun* had 1.0×10^8 . Maximum counts were recorded on the fifth day as 2.4×10^9 cfu/ml for modified *Omidun*, 7.2×10^9 cfu/ml for raw *Ogi* and refrigerated *Omidun* had 2.0×10^6 cfu/ml. Over the fifth

day, there was a decline in the counts of LAB in all the three fractions of *Ogi* used (Fig. 2).

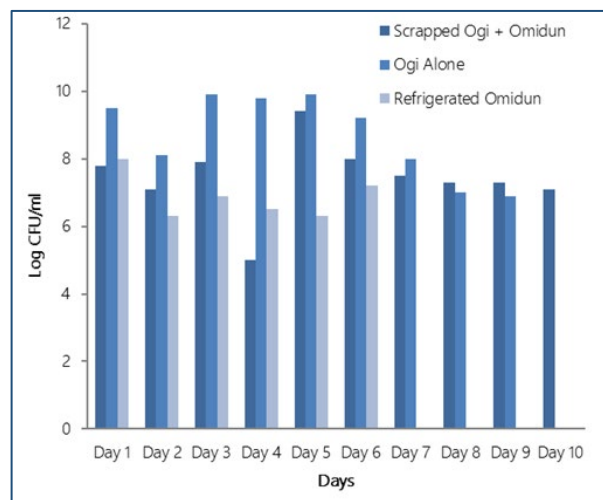


Figure 2: Viability of LAB in modified *Omidun*, refrigerated *Omidun*, and *Ogi* for 10 days

The effect of freeze-drying on the viability of *L. pentosus* and *L. paraplantarum* using *Ogi* and milk as cryoprotectants was reported on Fig. 3. Milk is a better cryopreserving agent than *Ogi*. For LAB strains cryopreserved in *Ogi*, there was a 3 log reduction in the cfu/ml after freeze-drying in both tested strains while for LAB strains cryopreserved in milk, there was only a slight reduction in the viability of two LAB, from 2.6×10^{12} to 1.1×10^{12} for *L. paraplantarum* and from 2.32×10^{12} to 6×10^{11} for *L. pentosus*. In freeze-dried *Ogi*, the reduction in viable cells was four logs from 5.2×10^9 to 7.2×10^5 cfu/ml (cf. Fig 3). Also, the effect of two months of storage at room temperature, on the viability of freeze-dried *L. pentosus* and *L. paraplantarum* cryopreserved in *Ogi*, milk, and *Ogi* alone, was reported. The viable LAB in *Ogi* alone reduced from 7.2×10^5 cfu/ml to 1.3×10^2 cfu/ml on day 26. There was two log reduction in the viability of *L. pentosus* cryopreserved with *Ogi* (not preserved in capsules) between day 1 and day 69 (from 2.77×10^8 to 2.9×10^6 cfu/ml) while there was a 3-log reduction in the viability of *L. paraplantarum* cryopreserved with *Ogi* (not preserved in capsules) (from 3.04×10^8 to 1.32×10^5 cfu/ml) (cf. Fig. 3). There was a statistically significant difference in the viability of LAB cryopreserved with milk versus *Ogi* ($p=0.012$).

The effect of 3 weeks of capsulation on freeze-dried strains cryopreserved in *Ogi* was reported. There was a drastic reduction in the viability of *Ogi* capsulated products at the end of 3 weeks to $<10^4$ cfu/ml in both strains, thereby leading to discontinuation of the experiment. However, the uncapsulated *Ogi* freeze-dried *L. paraplantarum* strain had a three-log reduction from 3.04×10^8 cfu/ml to 1.62×10^5 cfu/ml and a one log reduction for *L. pentosus* (from 2.77

$\times 10^8$ to 7×10^7 cfu/ml) (cf. Fig. 4). The milk capsulated products survived till the 69th day, but at reduced viability. For milk+ *L. paraplantarum*, there was a 6 log reduction (from 1.1×10^{12} to 3.7×10^5) and for milk+ *L. pentosus*, there was a 5 log reduction (from 6×10^{11} to 1×10^6) after 69 days (cf. Fig. 3).

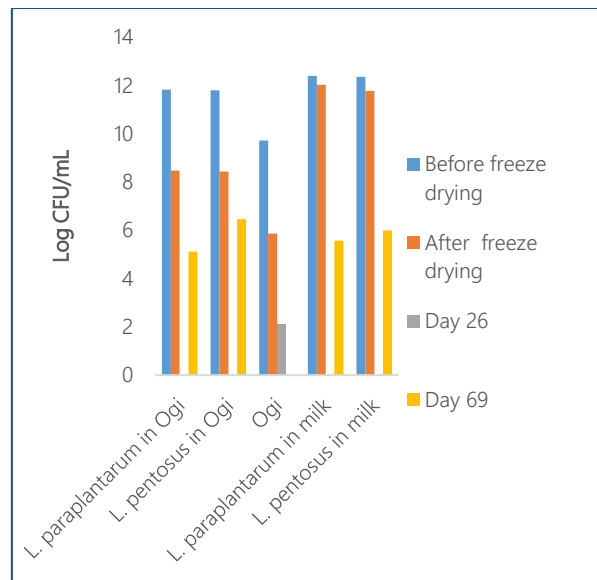


Figure 3: Effects of freeze-drying and storage on Lactobacilli strains

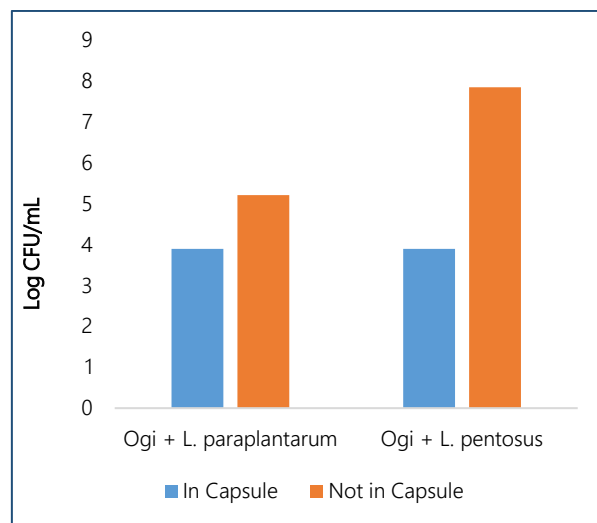


Figure 4: Comparison of the viability of Lactobacilli strains in freeze-dried *Ogi* after three weeks of capsulation and uncapsulation

The antimicrobial effect of modified *Omidun* against five strains of diarrhoeagenic *E. coli* was reported. For the control experiments, the *E. coli* strains either maintain, lower or increased its viability after incubation in saline for 24 h e.g. the log viability of EAEC (3 logs), EHEC (5 logs) and STEC (6 logs) were maintained at 0 and 24 h, EPEC increased from 4 logs to 5 logs while ETEC reduced from 6

to 5 log count. STEC was the most susceptible *E. coli* strain as no strain survived in *Omidun* after 24 hours of incubation. There was a 7 log reduction in ETEC strain, 4 log reduction in EAEC and 2 log reduction in EIEC. *Omidun* had little effect in reducing EPEC viability (cf. Fig 5).

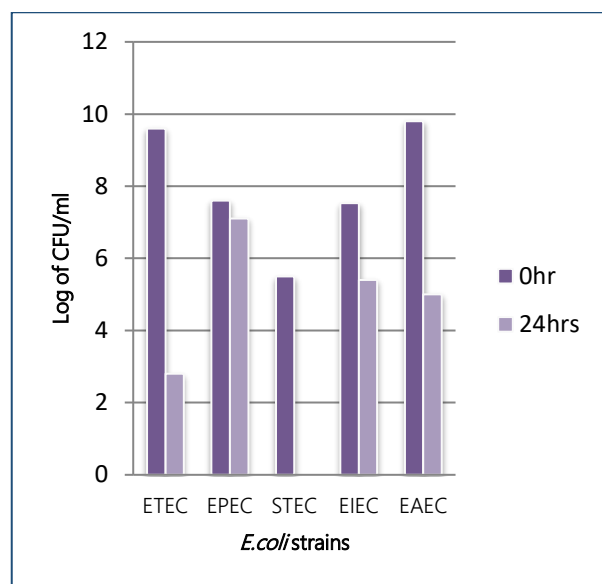


Figure 5: Antimicrobial Effects of Modified *Omidun* on Diarrheagenic *E. coli* strains

4 Discussion

This study reported that scrapping the surface of *Ogi* and mixing it with *Omidun* had a higher quantity of viable beneficial LAB in comparison to normal *Omidun* and freeze-dried *Ogi* with appropriate anti diarrheagenic *E. coli* activities thereby implying functional food ability of *Ogi*. We also report complete absence of LAB in *Ogi* after 10 days of milling, but rather a succession of fungi and yeast, thereby suggesting that the shelf life of *Ogi* is within 10 days if they are to be used as a functional food. *Ogi* could be consumed strictly as food with no consideration for attendant health benefits. However, if *Ogi* is to be considered as functional food with special interest in the naturally occurring beneficial bacteria, then, the viability of the bacteria is significant as probiotics, defined as live bacteria which when administered in adequate amount confer health benefits on the host. Therefore, viability of bacteria in functional food constitutes a key consideration.

The *Ogi* slurry used in the current study showed a progressive increase in the LAB population for a duration of 10 days after which there was a succession of fungi with no viable LAB. A repeated experiment confirmed that the viability of LAB in *Ogi* is only within 10 days. The occurrence of LAB in *Omidun* was reported by George and Anosike [17], where viable LAB was isolated from *Omidun* and the increase in population of LAB is supported by Afolayan *et*

al., [14] where the LAB growth in the *Ogi* slurry increased during 48 h of the souring period. However, to the best of our knowledge, this is the first study reporting a 10 days period for detecting viable LAB in *Ogi*.

The counts of LAB in the modified *Omidun* and *Ogi* were more than that of refrigerated *Omidun* with peak viability on the fifth day. This can possibly be attributed to fermentation attaining its peak on the fifth day. Subsequently, there was a decline in the count of LAB since it was assumed that the LAB were the major agents of fermentation. The lower counts of LAB recorded for refrigerated *Omidun* may have been due to the fact that refrigeration prevented fermentation from occurring. This is in contrast with the results reported by Afolayan *et al.*, [14] who recorded higher counts of LAB in *Omidun* than in raw *Ogi* and this may be due to the fact that the *Omidun* in their study was stored at room temperature which was not the case in this study. There have been reported of peak LAB count and after which, there was a decline in the counts of the viable LAB [14,18]. These findings are indicative of the fact that the normal preparation of *Ogi* or a mixture of raw *Ogi* and *Omidun* contain high quantities of LAB until the fifth day when stored at room temperature and the water is constantly changed.

The reduction in LAB counts in *Omidun* and increases in *Ogi* slurry can be attributed to the gravitational pull of the LAB from the *Omidun* to the *Ogi* surface. Therefore, a modified *Omidun*, used in our study, involves the scrapped surface of *Ogi* mixed with *Omidun* and it displays a high count of viable LAB. The modified *Omidun* viable count was higher than traditional *Omidun* because it combines viable counts in *Omidun* with the densely populated surface of *Ogi* where gravitational force has pulled down the bacteria. Aiming to provide health benefits, it will be essential that there is a minimum of 10^6 cfu g⁻¹ viable probiotic organisms in a product [14] or 10^7 cfu g⁻¹ at point of delivery [19]. Therefore, observed high viability of LAB in different components of *Ogi* is interesting.

In formulating freeze-dried products, the cryoprotectant has to be considered and it is essential that viability is maintained throughout the process of formulation and subsequently throughout its use. During freeze-drying, the cells are exposed to an extreme temperature that has the ability to damage the cells of the bacteria [20]. Cryoprotectants can then be used in optimizing this process, protect the cells and in the process enhance the viability of the organisms during freeze-drying [20]. As observed in this study, *Ogi* is not a suitable cryoprotectant during freeze-drying and capsule storage in comparison to using skimmed milk. The LAB, in formulation with milk, possesses a higher survival rate than LAB in *Ogi*

formulation. Ayeni *et al.* [16] reported that milk is highly effective in protecting the organisms during freeze-drying and enhancing the survival of the organisms during storage. This agrees with the study carried out by Jalali *et al.* [20] who reported 20% increase in viability of the organisms using 6% skimmed milk and the highest survival after 3 months in capsules that formulation is with sodium ascorbate and trehalose. Freeze-dried techniques have the advantage of being a process in which bacteria can survive well with the addition of cryoprotectants [16,20,21]. Temperature fluctuation is one of the factors that contribute to the survival and activity of LAB in a food sample [22]. The freeze-dried process has the advantage of preserving the LAB for a long time and also reduces the rate of destruction by the gastric acid due to the ease of micro-encapsulating a freeze-dried product.

Escherichia coli, which has been implicated as one of the major causes of diarrhea in humans, and the major cause of mortality and morbidity in children less than 5 years, has shown multi-resistance to antibiotics. The resistance of diarrhoeagenic *E. coli* to antibiotics has been ascribed to the misuse or under-use of antibiotics most especially ampicillin, chloramphenicol and sulphamethoxazole-trimethoprim [23,24]. Fermented foods can have an inhibitory effect on the diarrhoeagenic *E. coli* which could be due to different mechanisms of action of the LAB present in the fermented food [5,25]. These inhibit the growth of the pathogenic organism and the inhibitory effect is supported by a decrease in pH hence increase in acidity of the environment. From the co-culture experiment, there was reduction in the viable count of the selected diarrhoeagenic *E. coli* strain after 24 h of contact time. Interestingly, after 24 h, STEC LLH74B was entirely inhibited and drastic inhibition was observed with the other diarrhoeagenic *E. coli* strains. Afolayan and Ayeni [26] also observed a decrease in the count of *E. coli* strain EKT004 after a co-culture with LAB isolated from *Ogi* with more inhibitory activity of LAB against *E. coli* strain EKT004 when compared with the activity of conventional antibiotics. These reports demonstrate antimicrobial activity of LAB in fermented food, especially *Omidun* which in turn suggests that this group of bacteria is able to confer health benefits on individuals consuming them. It may, therefore, be important to encourage the use of a mixture of *Omidun* and raw *Ogi* or raw *Ogi* for better results.

The observed activities could be due to inhibitory compounds produced by lactobacilli e.g. organic acids, diacetyl, hydrogen peroxide, nisin, lactic acid and bacteriocins [25,27,28]. George and Anosike [17] also isolated three LAB from *Omidun* and showed their antimicrobial effect on some test microorganisms and

Ayeni and Ayeni, [29] reported that inoculated enteric pathogen was inhibited by LAB after 24 - 48 h contact time.

Furthermore, the decrease in the pH contributes to the inhibitory effect of the *Omidun* on *E. coli*. There was a drastic change in the pH value with a decrease from 4.06 to 2.90, hence an increase in acidity. This drastic change in pH has been reported [14]. The effectiveness of *Lactobacillus* species against enteropathogenic bacteria has been reported [30,31]. Interestingly, *Omidun* doesn't only have antimicrobial properties, but it offers protection against colitis in a rat model [32]. This further clarifies the medicinal properties of *Omidun* as reported in our study.

5 Conclusion

The current study provides the scientific proof of the use of *Omidun* in the local treatment of diarrhoea due to its anti-diarrhoeagenic activities. Furthermore, we demonstrated that *Ogi* and *Omidun* are best consumed within 10 days of souring for maximal lactic acid bacterial viability and antimicrobial effects. We present a modified *Omidun* that involves lightly scrapping the surface of *Ogi* and mixing it with *Omidun* to get a higher quantity of viable beneficial LAB.

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